Programmed Cell Death in Pathogenic Fungi

Mark Ramsdale

School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, United Kingdom

Tel: +44(0)1392-269173
Fax: +44(0)1392-263700
E-mail: M.Ramsdale@exeter.ac.uk

Keywords: Antifungal drugs; *Candida albicans*; Apoptosis; Necrosis

Abstract

Greater understanding of programmed cell death (PCD) responses in pathogenic fungi may offer a chance of exploiting the fungal molecular death machinery to control fungal infections. Clearly identifiable differences between the death machineries of pathogens and their hosts, makes this a feasible target. Evidence for PCD in a range of pathogenic fungi is discussed alongside an evaluation of the capacity of existing antifungal agents to promote apoptosis and other forms of cell death. Information about death related signalling pathways that have been examined in pathogens as diverse as *Candida albicans*, *Aspergillus fumigatus*, *Magnaporthe grisea* and *Colletotrichum trifolii* are discussed.

1. Introduction

Programmed cell death appears to be a ubiquitous feature of living systems, and has been described in one form or another in the majority of phylogenetic lineages including eubacteria, protists, plants and animals [1-8]. The wide spread occurrence of PCD hints either at an ancient origin, or suggests that it is an aspect of the life of both unicellular and multicellular organisms which has evolved many times over. In either case, PCD plays an important part in the life-histories of most organisms including fungi [9,10].

Differences in the nature of death responses are often a reflection of basic differences in the cell biology of the organisms under consideration. It is now apparent however that at a deep-rooted level, related molecules are taking part in the cell death decisions of organisms as diverse as bacteria, yeast, plants, worms, flies and man. Much research has focused on this similarity, but when differences are found they should be celebrated, as they may, in the case of pathogenic organisms provide a new avenue of investigation that could be exploited in the design of drugs that fight infectious diseases of plants and animals.

The core features of the PCD responses in mammals are defined by a set of morphological and biochemical changes that are mediated by external (extrinsic) or internal (intrinsic) cell suicide programs. In the intrinsic death pathway, death signals induce the release of mitochondrial proteins, [11-13] resulting in an amplification of a caspase cascade,[11,14,15]. In the extrinsic pathway, signals mediated by death receptors of the TNF receptor superfamily activate the caspase cascade directly. Caspase independent

suicide pathways may also be initiated in response to stress (eg after exposure of cells to ROS) that involve the translocation of an apoptosis inducing factor (AIF) from the mitochondrion to the nucleus.

PCD is commonly associated with the fragmentation of nuclei and degradation of DNA which can be linked to the activity of a number of different nucleases [16-23]. PCD is also accompanied by a loss of phospholipid asymmetry that involves the translocation of phophatidylserine (PS) to the outer leaflet of the plasma membrane [24,25]. Such lipid bilayer rearrangments require the activation of non-specific bidirectional phospholipid flippases and floppases along with the inhibition of aminophospholipid translocases, or scramblases that normally recycle PS to the inner leaflet [26,27]. ATP-binding cassette transporters such as ABCA-1 and CED-7 have been implicated in transbilayer redistribution of PS [28]; perhaps of some significance therefore is the finding that homologues of such ABC-transporter enzymes in fungi have been implicated in antifungal drug resistance [29-31] though their roles in fungal cell death *per se* have yet to be explored.

2. Understanding fungal PCD has wide implications

Programmed cell death responses have now been described in a range of fungi [9,10] though the majority of studies are focused on the yeasts *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida albicans* [32-37]. Fungal cells dying under a range of conditions exhibit several markers characteristic of apoptosis, including the rapid exposure of PS at the outer cell membrane (revealed by annexin binding), the margination of chromatin in nuclei, nuclear fragmentation and the degradation of DNA (revealed by the TUNEL test). In many cases it has been shown that exposure of cells to cycloheximide prevents these death associated changes, indicating that the death response requires active protein synthesis (eg [38]).

Functional analyses of genes in yeast have revealed that there are some similarities at the molecular level between fungal apoptosis and apoptosis of higher eukaryotes, with the identification of homologues of caspase-like cysteine proteases [39], AIF [40] and Htr2A/Omi [41]. On the whole it is anticipated that the proteins responsible for fungal cell death will be sufficiently distinct from their mammalian counterparts to make drug therapies feasible since bioinformatic screens of fungal genomes have shown that many of the known components of higher organism apoptosis are missing or highly divergent at the amino acid level [42]. The discovery of PCD responses in the model pathogenic fungi *C. albicans* [36,37], *Aspergillus fumigatus* [43,44] and *Magnaporthe grisea* [45] raises the long-term possibility of developing novel antifungal drugs and fungicides that clear infections by activating fungal cell suicide. Identification of the endogenous molecular switches that trigger fungal apoptosis is of paramount importance if we are to achieve these aims.

3. Fungal pathogens of man, animals and plants

Fungi can affect human welfare by destroying crop plants [46] or by causing life-threatening diseases in immunocompromised individuals [47] - see Table 1 for a summary of the pathogenic fungal species considered in this review and the literature considered.

In humans the major threats to our health are posed by pleiomorphic fungi, ie those that can grow in yeast, pseudohyphal or filamentous growth forms. Most notable amongst the pleiomorphic fungal pathogens is *Candida albicans* and its close relatives (*C. dubliniensis*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*), as well as the more distant relative, *Candida glabrata*. The other major human pathogenic fungi that cause life-threatening disease are *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Paracoccidioides brasiliensis* and *Blastomyces dermatidis*. A number of additional human pathogens are now also emerging as significant threats to specific patient groups (particularly HIV-positive patients) including *Penicillium marneffei* [48] and *Pneumocystis jiroveci*, formerly known as *P. carinii* [49]. In addition to these pathogens, a large number of clinically important dermatophyte fungi (numbering in excess of 600) can cause both irritating and/or disfiguring superficial infections. Amongst these, *Malassezia globosa,* a yeast that causes dandruff, as well as more severe seborrheic dermatitis, affects more than 50% of the human population and may contribute to atopic eczema in sensitized patients [50,51]. *Trichophyton rubrum* and *T. mentagrophytes*, cause superficial skin infections most notably athletes foot, and are considered to be the second most common cause of skin infections after acne [52]

Candida albicans has become a molecular genetics work-horse for the study of pathogenicity, virulence and fungal development [53]. *Candida* species are typically commensal organisms, present on about 50% of the population at any one time [54]. Over a lifetime however, some 80% of women suffer from clinical *Candida* infections, and about 5% of these thrush infections can be recurrent, with some infections becoming resistant to antifungal therapy. In immuno-compromised individuals, *C. albicans* can produce mild but irritating, superficial infections of the oral and vaginal mucosa. In severely immuno-compromised patients, *C. albicans* can produce a disseminated systemic infection which if not treated effectively is associated with a high incidence of mortality. Systemic *C. albicans* infections typically occur in patients undergoing chemotherapy or organ transplantation and, depending upon the patient group, one-third to one-half of these infections are fatal. The incidence of infection amongst premature and small babies can be as high as 7%, and over half of these patients may not survive [55]. *C. albicans* is also the fourth most common hospital acquired infection and typically extends a patient's stay in hospital by an average of 30 d. The number of clinical *C. albicans* infections in UK hospitals has risen significantly in recent years [56], and the incidence of resistance to traditional antifungal therapies is high [57]. *Candida* infections are therefore both socially and economically devastating.

4. Major plant pathogenic fungi

Human pathogenic fungi that cause life-threatening disease represent a small fraction of the species that cause disease. Indeed, whilst the majority of fungi are benign, a large number produce diseases in plants

affecting crop yields and profit margins which can have serious consequences on both local and global scales.

Arable food production worldwide is mainly based upon four staple crops - rice, wheat, maize and potato [58]. All of these are subject to infection by a significant number of plant pathogenic fungi. Fungicides are vital for the control of plant diseases, which are estimated to cause yield reductions of almost 20% in the major food and cash crops worldwide [59].

Arguably at the top of the list of plant pathogenic fungi is the filamentous ascomycete *Magnaporthe grisea* (sexual state *Pyricularia oryzae*), the causative agent of Rice Blast Disease [46]. Annually, rice blast is responsible for a loss of between 10 and 30% of the rice harvest. Other plant pathogens that pose serious environmental and socioeconomic threats include *Tilletia indica* (Karnal bunt, [60]) and *Pucciniania kuehnii* (Sugar Cane Orange Rust [61]). *Cryphonectria parasitica* a basidiomycete pathogen causing Chestnut blight [62,63] and *Ophiostoma* spp another ascomycete (Dutch Elm disease [64]) have also caused major losses in forestry in recent years.

Overall fungi represent 30% of emergent infectious diseases (second only to viral infectious diseases at 47% [65]. Despite extensive breeding programmes for resistance to fungal pathogens, the shear numbers of fungal propagules in the environment and the ability of fungi to generate diversity through sexual and parasexual recombination often mean that control is limited to only a few seasons before new virulent strains arise.

Amongst the oomycetes, *Phytophthora infestans* (Potato late blight) is still the most important threat to potato production worldwide [66]. This pathogen which coevolved with wild potato (Solanum) species, was transported to Mexico from South America [67] from where it spread to cultivated crops worldwide. It was introduced into the USA in about 1840 and was subsequently transported to Europe where it decimated potato production, causing the Irish potato famine and the forced migration of five million people [68]. Blight was re-introduced from Mexico into the USA and Canada during the early 1990s [69,70] and outbreaks of blight continue to this day, causing devastating local and global epidemics potentiated by the emergence of virulent fungicide-resistant strains such as US-8 [71]. Other species of *Phytophthora* are the cause of major economic and environmental losses including *P. ramorum* in Europe (Sudden Oak death Syndrome [72] and *P. cinnamomi* in Australia (Jarrah Die-back [73]).

5. Fungi as pests / spoilage organisms

Fungi also affect our quality of life more indirectly by damaging / spoiling food-stuffs (which leads to both economic losses or the production of food-stuffs contaminated with health-threatening mycotoxins). Fungi can colonize our homes, workplaces and hospitals (damaging property) and following the production of copious quantities of airborne spora, induce allergic reactions that can in some case be life-threatening eg *Stachybotrys chartarum* [74] and *Aspergillus fumigatus* [75]. Reducing the economic and environmental threats posed by such contamination requires the treatment of foods or property with a range

of preservative agents that that can become less effective with prolonged use, and themselves may be considered harmful to our health or the environment.

6. Worldwide economic losses attributed to fungal disease

In 2006 the annual fungicide market aimed at arable crops was estimated to be $7.2 billion [173]). Such massive expenditure is however offset by the economic gains arising from the increase in yields / productivity which are thought to have netted the farming industry $12 billion dollars in additional revenue. At a local level the impact of losing a crop can however be devastating, even when the industry as a whole performs well. Currently the antifungal drug market is of comparable size, estimated to be about $11.9 billion in 2007 [174].

7. Current practice - antifungals and fungicides

The design and implementation of effective antifungal / fungicide therapies is complicated by basic similarities in the cellular organization of pathogenic fungi and their hosts. In a medical setting many antifungal drugs are quite toxic to patients; precluding their long-term use.

Current antifungal treatments used in healthcare largely target essential processes at the fungal cell surface, such as plasma membrane or cell wall biogenesis. Several distinct classes of antifungal drug are currently available for the treatment of clinical fungal infections. Novel antifungals are always under development, but the mainstays of treatment and the most widely used are azoles, polyenes, allylamines, 5-fluorocytosine (5FC) and the echinocandins [76].

The polyene antifungal amphotericin B (AmB) has been used clinically for over 30 years [77]. Formulating AmB with liposomes [78], and lipid complexes [79] has allowed the use of higher doses of AmB, which can be useful in the treatment of recalcitrant infections. The primary mode of action of AmB, in common with other polyene antifungals is thought to arise from its affinity for ergosterol. Integration of AmB into the cell membrane results in the formation of aqueous pores, which lead to altered plasma membrane permeability which is subsequently accompanied by loss of mono- and divalent cations leading to cell death [80]. The minimal inhibitory concentrations (MIC_{90}) of AmB for a variety of species of *Candida* range from 0.25-1 μg/ml [81], whilst the minimal fungicidal concentrations (MFC) may be up to 2 fold higher. The fungicidal action of AmB makes it useful in the treatment of systemic candidiasis, *Candida* meningitis and ophthalmitis [76] as well as infections that are traditionally regarded as resistant to azoles eg *C. krusei* and *C. glabrata*. Polyenes also have a strong affinity for host sterols, especially cholesterol [82], which is associated with a number of side-effects, most importantly renal toxicity precluding their use in long-term therapy [83]. Resistance to AmB can be intrinsic, with isolates of *Candida luscitaniae* commonly failing to respond [76]. Acquired resistance, though rare, has been described, and is linked to the selection of mutants that accumulate 3β-ergosta-7,22-dienol and 3β-ergosta-8-dienol, which is associated with a defect in sterol Δ5,6 desaturases [84,85]. Increased catalase activity

has also been found to offset the oxidative (ROS) damage that accompanies AmB treatment of fungal cells which is thought to contribute to its fungicidal properties [86].

Azoles were discovered in the 1960s and have been the mainstay of antifungal therapies for a number of years. Two classes of azole antifungal drug have been developed, N-1 substituted imidazoles (eg ketoconazole, clotrimizole) and triazoles (eg fluconazole, itraconazole). Azoles target the CYP51A1 cytochrome P450 required for the 14α-demethylation of lanosterol [87] and the Δ22-desaturase involved with the desaturation of ergosta-5,7-dienol [88]. Nitrogen in the imidazole or triazole rings bind to the haem iron of cytochrome P450, inhibiting its action. The interaction depends on the precise conformation of the enzyme, thereby affecting the range of species against which individual azoles are effective. Despite this, azoles do generally possess broad spectrum antifungal properties and are used in the treatment of *Candida* spp., Cryptococcal infections, *H. capsulatum*, *Coccidioides immitis* and dermatophytes. The incidence of resistance to front-line azole antifungals is however one of the major driving forces behind the need to develop new active agents.

Exposure of fungi to 5-Fluorocytosine (5FC) leads to its uptake by cytosine permeases. Once inside a cell, 5FC is deaminated to 5FU and then converted to a nucleoside triphosphate, which when incorporated into RNA causes miscoding. 5FU, itself is also converted to a deoxynucleoside, which can subsequently inhibit thymidylate synthase. 5FC is fungicidal, however its spectrum of activity is limited because of widespread resistance, which is greatest amongst *Aspergillus* and *Candida* species [89]. 5-FC is often used in combination therapy with AmB because of the rapid acquisition of resistance by treated infections. Combined therapies often show contradictory responses *in vitro*, but *in vivo* animal models of both candidiasis and cryptococcosis do generally respond well [90].

Allylamines were initially developed in the 1970's and include terbinafine, naftidine and structurally related compounds such as tolnaftate. The allylamines inhibit squalene epoxidase, the first enzyme in the committed stage of ergosterol biosynthesis. Allylamines function as reversible, non-competitive inhibitors of squalene epoxidase which leads to ergosterol depletion.

Terbinafine is fungicidal against filamentous dermatophytes, but is only fungistatic against *Candida* species. Co-treatment of cells with calcineurin inhibitors eg cyclosporin A or FK506 can alter this balance promoting cell death, potentially expanding the utility of this drug [91].

Echinocandins, a class of cyclic lipohexapeptides are the most recent additions to the arsenal of antifungal drugs. Echinocandins have been shown to act as non-competitive inhibitors of β-1,3 glucan synthase which is required for the synthesis of glucan polymers, a major component of the fungal cell wall. Caspofungin, the first commercially available form of echinocandin, displays fungicidal activity against *Candida*, *Aspergillus*, *Histoplasma*, *Coccidioides* and *Blastomyces*, however it lacks activity against *Cryptococcus* and many filamentous fungal pathogens. Micafungin is fungicidal against yeasts [92], but only shows fungistatic properties against aspergilli. Currently there are no reports of resistance developing against these drugs in a clinical setting.

Fungicides used in the treatment of plant disease are very diverse, targeted at both narrow and wide spectra of fungal pathogens. Sulphur containing compounds and strobilurins inhibit the electron transport chain, the latter blocking ubiquinol-cytochrome *c* oxidoreductase of the cytochrome *bc1* complex III [93]. Copper fungicides, dithiocarbamates, substituted aromatics and organophosphorous compounds inhibit the activities of a wide range of enzymes and are relatively non-selective. Benzimidazoles and phenylamides inhibit DNA and RNA synthesis respectively, whilst dicarboximides inhibit both. Cyclopropane carboxamide carpropramid and phenoxyamide (AC382042) both target scytalone dehydratase and therefore inhibit the synthesis of melanin [94,95]. Azole antifungals are also commonly used in agriculture - potentially with the risk of the development of resistance to such agents amongst clinical isolates.

8. Resistance to antifungals

Resistance of pathogenic fungi to traditional antifungal therapies is a perennial problem and in some clinical situations is alarmingly high [57] making the identification of novel targets for antifungal therapy of some urgency. Resistance to antifungal drugs has both clinical and microbiological components [76]. Successful therapy depends upon a number of factors that not only depend upon the activity of the antifungal therapy but also the pharmacokinetics of the drug in a patient, and the status of their immune system. Resistance can arise through inefficient / inappropriate dosing of patients, inappropriate selection of antifungals, or repeated exposure. In recent years there has been a significant shift towards the isolation of more resistant *Candida* species in hospitals such as *C. glabrata*. The administration of azole antifungals as a prophylactic treatment may be a significant contributor to this trend [96,97].

Ideally microbiological resistance will be predictive of clinical resistance, though this is often not the case. Some fungi are intrinsically resistant to specific classes of antifungal drug eg, fluconazole is highly efficacious against *C. albicans* and *C. parapsilosis*, but *C. glabrata* and *C. krusei* are less susceptible [76].

Low mammalian toxicity and environmental impact as well as low residues in food, and compatibility with integrated pest management programmes are very important features that are required of new antifungals. A balance between cost, potency and safety is a major goal for both the agrochemical and pharmaceutical industries developing new antifungal agents.

9. Examples of PCD induced in pathogenic fungi by antifungal agents

Liao *et al.* [98] examined physiological changes in *C. albicans* cells treated with AmB and described three patterns of death. Death was always accompanied by a drop in ATP level but could be subdivided on the basis of plasma membrane integrity and mitochondrial membrane potential. Later, Phillips et al. [36] found that AmB treated cells that were able to exclude propidium iodide and produced ROS corresponded to an apoptotic sub-population. Normal treatment of systemic candidiasis may therefore already reduce infection loads by initiating apoptosis.

Protoplasts of *A. fumigatus* treated with 0.25-1 μg ml^{-1} AmB stain positive with annexin V, indicating PS translocation to the outer surface of the plasma membrane and also display dsDNA breakage, detectable

with the TUNEL assay [99]. Propidium iodide staining (indicative of necrosis) is less than 20% at 0.25 and 0.5 μg ml^{-1} AmB, but increases to 85% at higher doses. Pre-incubation of cells with cycloheximide prevents the appearance of apoptotic markers, indicating that killing requires active translation. In contrast, cycloheximide is not able to prevent the formation of propidium iodide positive cells at 1 μg ml^{-1}. Taken together this suggests that at low fungicidal doses, AmB is pro-apoptotic and at higher doses it is pro-necrotic.

Pradimicin A, a broad spectrum fungicidal antifungal agent which binds to mannan residues in the cell wall [100], also induces apoptosis-like cell death in *S. cerevisiae* [101]. Nuclear fragmentation and DNA damage have been observed in yeast cells treated with Pradimicin A, accompanied by an accumulation of reactive oxygen species (ROS). Pradimicin-induced cell death and the accumulation of ROS are prevented by free radical scavengers, suggesting some dependency between the two.

Translational inhibitors such as the phenanthrolines have been shown to kill both mammalian and *C. albicans* cells [102]. Mammalian cells exposed to phenanthrolines show hallmarks of apoptosis, whilst *C. albicans* cells accumulate ROS and display elevated oxygen consumption rates. Nuclear disruption, including enlargement and formation of crescent shaped bodies, has been observed after treatment of yeast cells with some (though not all) silver, copper and manganese metal 'phen' complexes The production of ROS in the absence of DNA damage could imply that ROS play a primary role as a signal of apoptosis and do not act directly as the DNA damaging agents. Killing by these translational inhibitors may involve a reduction in the levels of cytochrome *b* and *c* and the associated uncoupling of respiratory function which might contribute to the formation of pro-apoptotic ROS. McCann et al. [103] found that phenanthrolines lowered the ratios of reduced:oxidised glutathione, consistent with a pro-oxidant role for the effects of these drugs.

A number of natural antifungal proteins have been found to exert their killing effect by induction of apoptosis like cell death. Osmotin, a member of the PR-5 family of plant defence proteins isolated from tobacco [104]; the basic, cysteine-rich antifungal protein PAF from *Penicillium chrysogenum* [105]; Dermaseptins from amphibian skins [106,107] and virally encoded yeast killer toxins [108] all appear to induce apoptosis in fungi. RsAFP2 an antifungal peptide isolated from *Raphanus sativus* interacts with glucosylceramides in membranes of fungi resulting in the production of ROS and the death of *C. albicans* cells [109]. Salivary histatins, short histidine rich peptides have also been shown to display both fungistatic and fungicidal activity against a range of *Candida* species[110]. Wunder et al.[110] concluded that histatins did not induce apoptosis in *C. albicans* since they could not find any evidence of DNA laddering or the release of cytochrome *c* when isolated mitochondria were treated with histatin 5. Although elevated levels of ROS were detected following the exposure of cells to histatin 5 or the intracellular expression of an Hst 5 construct, the reduction in viability of *SOD1/2* mutants of *S. cerevisiae* or *SOD1* mutants of *C. albicans* was the same as wild type strains leading the authors to conclude that ROS production merely accompanied the death response, rather than contributing significantly to its fungicidal activity., The significant delay in killing that occurs after treatment has begun does still suggest that histatins might induce some form of

PCD [36, 111]. Indeed, whilst the results of Wunder et al. [110] indicate that the primary mode of action of histatins is not the release of cytochrome *c* from mitochondria, the failure to detect PCD might be due to the fact that canonical markers of apoptosis were not examined in intact cells.

Some antifungal drugs in some species do however appear to operate quite independently of apoptotic mechanisms. For example treatment of protoplasts of *A. fumigatus* with itraconazole or *A. nidulans* with aureobasidin A, AmB or itraconazole does not induce apoptosis [99, 112]. Furthermore, fluconazole toxicity has been shown to be independent of known apoptotic mechanisms in yeast, though this was only tested in the context of a failure of heterologously expressed Bcl-2 to inhibit killing and death characteristics *per se* were not addressed [113].

Finally, radio-labelled monoclonal antibodies have been used to treat *C. neoformans* and *H. capsulatum* infections [114]. An examination of the susceptibility of these species to ^{213}Bi and ^{188}Rd indicated that whilst both species were quite resistant, doses over 4 kGy produced apoptotic changes and killing.

10. Studies of PCD in pathogenic fungi

Direct studies of apoptosis-like cell death in pathogenic fungi have given us some insights that are not possible when looking at model fungi such as *Saccharomyces cerevisiae* in isolation. For example isolates of *Colletotrichum gloeosporioides*, a pathogen of the weed *Aeschynomene virginica*, display enhanced longevity when expressing the anti-apoptotic Bcl-2 protein [115]. Cells are also protected from Bax-induced cell death, and exhibit enhanced stress resistance - all generally consistent with similar experiments in yeast [116]. However the isolates also show enhanced mycelium production and conidiation, and are hypervirulent to host plants. The endogenous apoptosis-related cell machinery may therefore be important for regulating morphogenetic switches, which are critical for proper responses and adaptation of fungal pathogens to different environments [10].

C. albicans activates a PCD response (with features reminiscent of apoptosis and necrosis) in response to a variety of environmental stimuli such as acetic acid, hydrogen peroxide as well as AmB [36]. This fungal PCD response is characterised by the rapid appearance of several classical apoptotic markers observed in mammalian cells including a loss of cell viability accompanied by the exclusion of the vital dye propidium iodide; sustained oxygen consumption and metabolic activity during cell death; the production of ROS in apoptotic cells (indicated by oxidation of dihydrorhodamine); the condensation of chromatin at the nuclear margin (visible with DAPI staining and TEM) and the accumulation of DNA breaks (as revealed by TUNEL positive staining). The exposure of PS on the outer surface of the plasma-membrane (as revealed by annexin-FITC labelling) has also been observed. In late stages of cell death, cells lose their ability to exclude propidium iodide linked with the onset of secondary necrosis. Currently, we know very little about the effector molecules that are associated with the onset of PCD in pathogenic fungi. Using the power of functional genetics in fungi it has been possible to ascertain the extent to which individual signalling pathways are necessary, or sufficient for PCD - taking us a step closer to the possible development of antifungal drugs that stimulate PCD.

11. PCD related signalling pathways in pathogenic fungi

Biella et al. [117] reported that the response of the chestnut blight fungus *Cryphonectria parasitica* to infection with a viral dsRNA hypovirulence factor resembled PCD. Using microarrays to look for genes that were differentially regulated following infection with the virus, 295 sequences (out of 2,200) were found with changed abundance [118]. Using differential display, Chen et al. [119] observed that 65% of the global changes initiated by viral infection could be reproduced by manipulating G-protein and cAMP signalling pathways – indirectly supporting a link between death responses and Ras-cAMP-PKA activity in fungi.

Direct evidence of Ras-cAMP-PKA involvement in fungal cell death responses comes from studies of a number of pathogens including *Colletotrichum trifolii* (a pathogen of alfalfa) and *C. albicans*. In *C. trifolii*, expression of a hyperactive oncogenic fungal Ras protein (DRas) elevates the production of ROS leading to abnormal fungal growth and apoptosis-like cell death when grown under nutrient deprived conditions [120]. Addition of antioxidants such as N-cysteine, diphenylene iodonium, or proline can however rescue cells from undergoing apoptosis. Proline was found to have a general suppressive effect as a ROS scavenger, perhaps mediated by an increased level of catalase activity in addition to a well characterized role as an osmolyte.

In *C. albicans,* mutations that block Ras-cAMP-PKA signalling (*ras1*Δ, *cdc35*Δ, *tpk1*Δ, *tpk2*Δ) suppress or delay the apoptotic response induced by weak acid exposure [121]. In contrast mutations that stimulate signalling (*RAS1*^{val13} or *pde2*Δ) accelerate the rate of entry of cells into apoptosis when cells are treated with low doses of weak acid. Pharmacological stimulation of the Ras-cAMP-PKA pathway (either with dibutyryl cAMP, caffeine, or forskolin) enhances killing, whilst inhibition of Ras with lovastatin reduces apoptotic cell death. Transient increases in endogenous cAMP occur under conditions that stimulate apoptosis, but not stress or growth arrest indicating that there may be a separation of the activity of the Ras-cAMP-PKA pathway under stress and death inducing conditions.

Studies of the response of *S. cerevisiae* cells treated with the plant defence molecule osmotin, shed more light on this idea, since the production of ROS, expression of antioxidant proteins and the apoptotic response are partially dependent upon an induced suppression of the *RAS2*/cAMP pathway [104]. *RAS2*^{G19V}, a dominant active allele of Sc *RAS2*, increases sensitivity of cells to osmotin and a null mutant shows reduced sensitivity. The response can be linked specifically to the Ras-PKA rather than the Ras-MAPK signalling pathway because the effects of the dominant active allele are also seen in a *ste20* background. Consistent with osmotin induced Ras-PKA signalling, a *bcy1* null, with constitutively active PKA activity, exhibits significantly increased sensitivity to osmotin. De-repression of STRE-dependent transcriptional responses in *ras2* mutants [122] might account for the elevated resistance of *RAS2* nulls to stress treatments - a view further supported by the finding that during osmotin induced PCD, both STRE-element and YRE-reporter constructs are repressed [104]. It might therefore be argued that the balance between stress and apoptotic signals determines cell fate, and that osmotin stimulates pro-apoptotic ROS

production via the activation of the RAS2/cAMP pathway which in turn inhibits YRE (Yap1-dependent) and STRE-mediated antioxidant stress response

Over-expression of plant defence molecules induces a hyper-branching phenotype or the formation of spiral hyphae [104] and growth inhibition *per se* has long been linked to altered patterns of hyphal branching [123, 124]. Ras-signals have been linked to morphogenesis in a number of fungi (see [125] for a review), however a study of the link between death and morphogenesis in *C. albicans* showed that it could not be attributed to any of the known signalling pathways (*EFG1*, *RIM101*, *TEC1*, *CPH1*) that contribute to morphogenesis [36]. Morphogenesis could however require the integration of many signals and pathways, including Ras under the specific conditions examined.

G-protein signals have been linked to apoptosis of *Aspergillus nidulans* induced by osmotin and the antifungal protein PAF, a small basic cysteine rich antifungal protein produced by *Penicillium chrysogenum* [105]. PAF-treatment induces hyperolarization of the cell membrane which is accompanied by PS exposure, ROS production and a TUNEL positive phenotype. A dominant-interfering mutant of *fadA*, which leads to constitutive inactivation of heterotrimeric G-protein signals [126] confers resistance to both osmotin [127] and PAF [105].

Few studies of pathogenic fungi have looked at signalling pathways other than Ras. However, several lines of evidence suggest that Ca^{2+}/calmodulin/calcineurin signals might affect the fungal death response. In *S.cerevisiae* apoptosis induced by pheromone treatment and salt stress have been shown to be influenced by mutations in calmodulin / calcineurin signalling pathway [128,129]. In addition, azole activity against *S. cerevisiae* is reduced by the addition of Ca^{2+} and enhanced by the addition of EGTA [130]. Inhibitors of the Ca^{2+} binding regulatory protein calmodulin such as fluphenazine, calmidazolin and W-7, as well as inhibitors of Ca^{2+}-dependant calmodulin-regulated phosphatase, calcineurin (cyclosporin and FK506) enhance azole activity. Consistent with these findings mutations that constitutively activate calcineurin demonstrate reduced azole susceptibility. When *CRZ1* (a transcription factor regulated by calcineurin) is disrupted cells also show enhanced azole sensitivity - clearly indicating that the cell integrity pathway is important for the action of these drugs.

Sanglard et al. [131] reported that FK506 treatment of fluconazole treated *C. albicans* cells induced a fungicidal, rather than a fungistatic response, which could have very important ramifications for future drug therapy regimens. Deletion of *CYP1* prevented the fungicidal activity, implying that a cyclophilin was essential for fluconzaole toxicity. A similar effect of FK506 has recently been reported in *Aspergillus fumigatus* when cells are treated with caspofungin or nikkomycin Z [132].

Using nested, iterative PSI-BLAST searches, Uren et al. [133] identified a family of caspases, metacaspases and paracaspases in plants, animals and fungi. In fungi several metacaspases have been found; whilst *S. cerevisiae*, *C. albicans, S. pombe* and have single metacaspase encoding genes (*YCA1*/*MCA1* and *PCA1* respectively), *A. fumigatus*, *A. nidulans* and *N. crassa* appear to have two each [99,112,134,135]. Many studies have now shown that apoptosis in yeast may be dependent upon the

activity of the metacaspases [39, 136-138]. In some scenarios however the apoptotic killing response does appear to be metacaspase independent [40,41,139,140].

To date experimental studies of the role of metacaspases in the cell death response of pathogenic fungi have been limited to *Aspergillus fumigatus* [43,44]. Stationary phase cultures of *A. fumigatus* exhibit strong intracellular activity against substrates specific for caspase-1 and -8 and the development of an apoptotic phenotype is blocked by Z-FAD-fmk. However deletion of both *casA* and *casB*, the two genes encoding the mtacaspases, had little effect on measurable caspase activity, viability of hyphae or pathogenicity [44]. PS-exposure in the double knock-out strain was strongly abrogated, leading Richie et al [44] to explore other possible functions for the *casA* and *casB* genes, including a role in endoplasmic reticulum homeostasis.

Other "non-classical" cell death pathways may be linked to the death of fungal cells and tissues. The development of turgor pressure in appressoria in the rice-blast fungus *Magnaporthe grisea* is a prerequisite for pathogenicity [141,142] and recently this process has been shown to be reliant upon the autophagic death of the germinating spore [45]. Moreover, knock-out mutants of Mg*ATG*8 arrest conidial death, and prevent pathogenicity, showing that the blocking of a fungal cell death response, rather than its stimulation, can also lead to control. Blocking of fungal PCD might also be useful is the control of many other fungi, including the economically important rust fungi. Whilst there have been no overt studies of PCD in rusts, it is evident that in some situations the death of support cells in rusts is essential for the dispersal of others [143]. Specifically terminal aeciospores are normally separated by dead suspensor cells. Clearly prevention of this step (blocking death) could prevent dissemination of this important group of pathogens.

12. Genetic screens for potential antifungal drug targets

In an ongoing exploration, some 30 fungal genomes have been fully sequenced, which currently includes 18 hemiascomycetes, 8 euascomycetes and 4 basidiomycetes [144]. In total, 17 of these sequenced genomes are from fungal pathogens, with the long term prospect that many more will be sequenced in the future. This enormous bioinformatic resource is now being explored by the pharmaceutical and agrochemical industries in the search for genes, conserved between species, that might be useful targets for future antifungal drug therapies. Potential drug targets are typically prioritized in terms of their degree of essentiality, broad-spectrum potential, drug target potential, fungal specificity, availability of functional assays, and amenability to high-throughput screening [145].

Recognizing the need for broad-spectrum antifungals, Liu et al [145] identified 240 putative antifungal targets that were conserved among 10 fungal species in order to develop a system to identify target-specific inhibitors. Essentiality for a selection of the genes was then determined in *C. albicans* using a repressible Ca*MET3* promoter. The value of this approach is that not only is the target known *a priori*, which helps with the subsequent phase I development of the drug or fungicide, the impact of down-regulating the gene can be directly assessed. The use use of dominant selectable markers (eg *NAT1* conferring resistance to nourseothricin) and *in vivo* regulatable doxycycline-sensitive promoters (eg the GRACE™ strain collection of *C. albicans,*[146]) is particularly attractive for the validation of the essentiality of targets prior to drug

screening. Such bioinformatic screens have yet to produce clinically useful drugs, though the potential is clearly there [147].

Expression of pro-apoptotic members of the BCL-2 family of proteins, Bax and Bak, have been shown to kill yeast cells, and cell death is typically associated with an apoptotic phenotype [148-150]. Expression of a codon-optimized *BAX* gene in *Candida albicans* has been found to result in growth inhibition and cell death. By fusing Bax with GFP, the cell death-inducing effect of Bax was increased due to reduced proteolytic degradation of the Bax protein [151]. However, not all fungi respond in the same way to heterologous pro-apoptotic proteins, with expression of Bax in *Pichia pastoris* leading to growth arrest accompanied by the condensation of chromatin, and the accumulation of autophagic bodies [152], but no other apoptotic features.

In the search for novel antifungal drug targets we shouldn't just restrict our search to essential genes, or to studying the effects of the expression of heterologous pro-apoptotic proteins since the over-expression of many endogenous genes can also lead to growth arrest / cell death. Such genes are involved with many different biological processes but notably include components of the cytoskeleton [153-156] and a variety of signal transduction pathways [157-161]. In genome wide library screens using cDNA or genomic clones, lethal effects have been observed for *ABP1*, *ACT1*, *ARF2*, *ATE1*, *AUA1*, *BIK1*, *BNI1*, *BOI1*, *ERG6*, *GCL17*, *HSF1*, *KAR1*, *MCM1*, *NHP6A*, *NHP6B*, *NPS1*, *NSR1*, *NTH1*, *PRK1*, *PSP1*, *RBP1*, *RHO1*, *STE4*, *STE11*, *STE12*, *SAC7*, *SEC17*, *SIR1*, *SNU114*, *SRP40*, *TPK1, TPK3, TUB1* and *URA2* [162-165]. Whilst the induction of PCD has not been explicitly studies in these investigations, it is intriguing to note that both *BNI1* (Gin2) and *BOI1* (Gin7) produce cells with multiple DAPI staining bodies - perhaps hinting at evidence of nuclear fragmentation and apoptosis [165]. Often death in an over-expression screen will not due to the increased activity of a dedicated pro-death protein, but due to an imbalance in some unrelated critical process, nevertheless, such screens could provide a useful starting point to look for pro-death functions.

The majority of studies of essential genes, or genes that when over-expression prevent growth, have not so far discriminated between the responses that are lethal and those that merely cause growth arrest. It may therefore be of considerable value to ascertain the degree to which shutting off/inducing gene expression induces killing, and of course by what route. Performing screens under conditions that stimulate PCD may also allow antagonists of PCD to be identified that have anti/pro-apoptotic properties. Indeed, we can speculate that many of the ‘essential’ genes that have been described in fungi may have such anti-apoptotic roles.

A final area that could be explored further in the search for novel antifungals that stimulate fungal PCD relates to the response of a fungal pathogen to its host during infection, in particular the events that accompany its clearance by the host immune system (plant or animal). *C. albican*s infections are controlled in immuno-competent individuals through the activity of both the innate and adaptive immune system; indeed defects in the innate immune system are often responsible for predisposing patients to disseminated disease. Macrophages and neutrophils provide some of the primary lines of defence,

consequently their interaction with *C. albicans* cells has been the subject of a large number of studies (see review in [166]). It is apparent that the interaction results in a major re-organization or re-programming of the transcriptional activity of both the host [167-169] and fungal cells [170, 171]. Recently Fernandez-Arenas et al. [172] produced a model, based upon combined proteome and transcriptome data obtained from *C. albicans* cells ingested by RAW264.7 macrophages, indicating that the changes observed in the actin cytoskeleton and mitochondrial functioning could be associated with the onset of two distinct pathways of killing; autophagic death or apoptosis. Clearly, further work needs to be undertaken to unravel the nature of the killing mediated by immune cells, but the information obtained could prove to be very useful in designing therapies that manipulate the delicate balance between a pathogen and its host.

Conclusion

This review has examined what little we do know about the cell biology of death responses in pathogenic fungi and shows how a combination of cell biological approaches, functional genetic analyses, genetic screens and global profiling technologies may just begin to unravel this important, but neglected, aspect of fungal growth and development. Furthermore, the existence of a number of discrete endogenous cell suicide pathways in fungi might be usefully exploited in the search for and design of novel therapies in the future.

Acknowledgements

The author wishes to apologize to any researchers whose relevant work was not cited or discussed in this review. Funding for this work was supported by grant funding from the BBSRC (BB/C501176/1).

References

[1] M.B.Yarmolinsky, Programmed cell death in bacterial populations, Science 267 (1995) 836.
[2] A. Hochman, Programmed cell death in prokaryotes, Crit Rev Microbiol 23 (1997) 207.
[3] H. Engelberg-Kulka, G. Glaser, Addiction modules and programmed cell death and anti-death in bacterial cultures, Annu Rev Microbiol 53 (1999) 43.
[4] S. Matsuyama, S. Nouraini, J.C. Reed, Yeast as a tool for apoptosis research, Curr. Opin. Cell Biol. 2 (1999) 618.
[5] K. Lewis, Programmed Death in Bacteria, Micro. Mol. Biol. Rev. 64 (2000) 503.
[6] E. Lam, Controlled cell death, plant survival and development, Nat. Rev. Mol. Cell Biol. 5 (2004) 305.
[7] J.C. Ameisen, The origin of programmed cell death, Science 272 (1996) 1278.
[8] P. Golstein, L. Aubry, J.P. Levraud, Cell-death alternative model organisms: why and which? Nat. Rev. Mol. Cell Biol. 4 (2003) 798.
[9] B.C.K. Lu, Programmed Cell Death in Fungi . The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. Growth, Differentiation and Sexuality, 2nd Edition (Eds Ursula Kües and Reinhard Fischer). Springer Berlin Heidelberg, 1 (2006) 167.

[10] M. Ramsdale, Programmed cell death and apoptosis in fungi. The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. Fungal Genomics, 1st Edition (Eds A.J.P. Brown). Springer Berlin Heidelberg, 13 (2006) 113.

[11] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c, Cell 86 (1996) 147.

[12] C. Du, M. Fang, Y. Li, L. Li, X. Wang, Smac, a mitochondrial protein that permits cytochrome c-dependent caspase activation by eliminating IAP inhibition, Cell 102 (2000) 33.

[13] A.M. Verhagen, P.G. Ekert, M. Pakusch, J. Silke, L.M. Connolly, G.E. Reid, R.L. Moritz, R.J. Simpson, D.L. Vaux, Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins, Cell 102 (2000) 43.

[14] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479.

[15] H. Zou, W.J. Henzel, X. Liu, A. Lutschg, X. Wang, Apaf-1, a human protein homolog to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3, Cell 90 (1997) 405.

[16] M. Enari, H. Sakahira, H. Yokoyama, K. Okawa, A. Iwamatsu, S. Nagata, A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD, Nature 391 (1998) 43-50.

[17] X. Liu, H. Zou, C. Slaughter, X. Wang, DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis, Cell 89 (1997) 175-184.

[18] X. Liu, P. Li, P. Widlak, H. Zou, X. Luo, W.T. Garrard, X. Wang, The 40-kDa subunit of DNA fragmentation factor induces DNA fragmentation and chromatin condensation during apoptosis, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8461-8466.

[19] L.Y. Li, X. Luo, X. Wang, Endonuclease G is an apoptotic DNase when released from mitochondria, Nature 412 (2001) 95-99.

[20] J. Parrish, L. Li, K. Klotz, D. Ledwich, X. Wang, D. Xue. Mitochondrial endonuclease G is important for apoptosis in *C. elegans*, Nature 412 (2001) 90-94.

[21] J. Zhang, M. Xu. Apoptotic DNA fragmentation and tissue homeostasis, Trends Cell Biol. 12 (2002) 84-89.

[22] H. Lecoeur, Nuclear apoptosis detection by flow cytometry: Influence of endogenous endonucleases, Exp. Cell Res. 277 (2002) 1-14.

[23] P. Widlak, L.Y. Li, X. Wang, W.T. Garrard, Action of recombinant human apoptotic endonuclease G on naked DNA and chromatin substrates: Cooperation with exonuclease and DNase I, J. Biol. Chem. 276 (2001) 48404-48409.

[24] V.A. Fadok, D.R. Voelker, P.A. Campbell, J.J. Cohen, D.L. Bratton, P.M. Henson, Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages, J. Immunol. 148 (1992) 2207-2216.

[25] S.J. Martin, C.P. Reutelingsperger, A.J. McGahon, J.A. Rader, R.C. van Schie, D.M. LaFace, D.R. Green, Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: Inhibition by overexpression of bcl-2 and abl, J. Exp. Med. 182 (1995) 1545-1556.

[26] D.L. Bratton, V.A. Fadok, D.A. Richter, J.M. Kailey, L.A. Guthrie, P.M. Henson, Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase, J. Biol. Chem. 272 (1997) 26159-26165.

[27] S.C. Frasch, P.M. Henson, J.M. Kailey, D.A. Richter, M.S. Janes, V.A. Fadok, D.L. Bratton, Regulation of phospholipid scramblase activity during apoptosis and cell activation by protein kinase C delta, J. Biol. Chem. 275 (2000) 23065-23073.

[28] M.F. Luciani, G. Chimini, The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptotic cell death, EMBO J. 15 (1996) 226-235.

[29] D. Sanglard, F. Ischer, M. Monod, J. Bille, Susceptibilities of *Candida albicans* multidrug transporter mutants to various antifungal agents and other metabolic inhibitors, Antimicrob. Agents Chemother. 40 (1996) 2300-2305.

[30] D. Sanglard, F. Ischer, M. Monod, J. Bille, Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: Characterization of CDR2, a new multidrug ABC transporter gene, Microbiology 143 (Pt 2) (1997) 405-416.

[31] S. Perea, G. Gonzalez, A.W. Fothergill, W.R. Kirkpatrick, M.G. Rinaldi, T.F. Patterson, In vitro interaction of caspofungin acetate with voriconazole against clinical isolates of *Aspergillus* spp, Antimicrob. Agents Chemother. 46 (2002) 3039-3041.

[32] F. Madeo, E. Frohlich, K.U. Frohlich, A yeast mutant showing diagnostic markers of early and late apoptosis, J. Cell Biol. 139 (1997) 729-734.

[33] F. Madeo, E. Herker, S. Wissing, H. Jungwirth, T. Eisenberg, K.U. Frohlich, Apoptosis in yeast, Curr. Opin. Microbiol. 7 (2004) 655.

[34] W.C. Burhans, M. Weinberger, M.A. Marchetti, L. Ramachandran, G. D'Urso, J.A. Huberman, Apoptosis-like yeast cell death in response to DNA damage and replication defects, Mutat. Res. 532 (2003) 227-243.

[35] Q. Zhang, H.K. Chieu, C.P. Low, S. Zhang, C.K. Heng, H. Yang, *Schizosaccharomyces pombe* cells deficient in triacylglycerol synthesis undergo apoptosis upon entry into the stationary phase, J. Biol. Chem. 278 (2003) 47145-47155.

[36] A.J. Phillips, I. Sudbery, M. Ramsdale, Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*, Proc. Natl. Acad. Sci. USA 100 (2003) 14327-14332.

[37] Y-U. Baek, Y-R. Kim, H-S. Yim, S-O. Kang, Disruption of γ-glutamylcysteine synthetase reults in absolute glutathione auxotrophy and apoptosis in *Candida albicans*, FEBS Lett 556 (2004) 47-52.

[38] P. Ludovico, M.J. Sousa, M.T. Silva, C. Leao, M. Corte-Real, *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid, Microbiology 147 (2001) 2409-2415.

[39] F. Madeo, E. Herker, C. Maldener, S. Wissing, S. Lachelt, M. Herlan, M. Fehr, K. Lauber, S.J. Sigrist, S. Wesselborg, K.U. Frohlich, A caspase-related protease regulates apoptosis in yeast, Mol Cell 9 (2002) 911-917.

[40] S. Wissing, P. Ludovico, E. Herker, S. Buttner, S.M. Engelhardt, T. Decker, A. Link, A. Proksch, F. Rodrigues, M. Corte-Real, K-U. Frohlich, J. Manns, C. Cande, S.J. Sigrist, G. Kroemer, F. Madeo, An AIF orthologue regulates apoptosis in yeast, J. Cell Biol. 166 (2004) 969-974.

[41] B. Fahrenkrog, U. Sauder, U. Aebi, The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis, J. Cell Sci. 117 (2004) 115-126.

[42] A. Fraser, C. James, Fermenting debate: do yeast undergo apoptosis? Trends Cell Biol. 8 (1998) 219-221.

[43] S.A. Mousavi, G.D. Robson, Entry into the stationary phase is associated with a rapid loss of viabiliy and an apoptotic-like phenotype in the opportunistic pathogen *Aspergillus fumigatus*, Fungal Genet. Biol. 39 (2003) 221-229.

[44] D.L. Richie, M.D. Miley, R. Bhabhra, G.D. Robson, J.C. Rhodes, D.S. Askew, The *Aspergillus fumigatus* metacaspases CasA and CasB facilitate growth under conditions of endoplasmic reticulum stress. Molec. Microbiol. 63 (2007) 591.

[45] C. Veneault-Fourrey, M. Barooah, M. Egan, G. Wakley, N.J. Talbot, Autophagic fungal cell death is necessary for infection by the rice blast fungus, Science 312 (2006) 580.

[46] N.J. Talbot, On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annu. Rev. Microbiol.* **57** (2003) 177.

[47] K.J. Kwon-Chung, J.E. Bennett, Medical Mycology, Lea & Febiger, Philadelphia, PA, (1992), pp. 142.

[48] N. Vanittanakom, C.R. Cooper, M.C. Fisher, T. Sirisanthana, *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects, Clinical Microbiology Reviews, 19 (2006) 95-110.

[49] A. Morris, J.D. Lundgren, H. Masur, P.D. Walzer, D.L. Hanson, T. Frederick, L. Huang, C.B. Beard, J.E. Kaplan, Current epidemiology of *Pneumocystis* pneumonia, Emerg. Infect. Dis. 10 (2004) 1713.

[50] J. Xu, C.W. Saunders, P. Hu, R.A. Grant, T. Boekhout, E.E. Kuramae, J.W. Kronstad, Y.M. DeAngelis, N.L. Reeder, K.R. Johnstone, M. Leland, A.M. Fieno, W.M. Begley, Y. Sun, M.P. Lacey, T. Chaudhary, T. Keough, L. Chu, R. Sears, B. Yuan, T.L. Dawson, Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens, Proc. Natl. Acad. Sci. USA 104 (2007) 18730-18735.

[51] P. Schmid-Grendelmeier, A. Scheynius, R. Crameri. The role of sensitization to *Malassezia sympodialis* in atopic eczema, Chem. Immunol. Allergy 91 (2006) 98-109.

[52] A.K. Gupta, J.E. Ryder, M. Chow, E.A. Cooper, Dermatophytosis: The Management of Fungal Infections, SKINmed 4 (2005) 305.

[53] F.C. Odds, A.J.P. Brown, N.A.R. Gow, *Candida albicans* genome sequence: a platform for genomics in the absence of genetics, Genome Biology 5 (2004) 230-235.

[54] F.C. Odds, Candida and Candidosis, Bailliere Tindall, London, (1988).

[55] V. Krcmery, M. Fric, M. Pisarcikova, M. Huttova, J. Filka, K. Kralinsky, H. Hupkova, J. Hanzen, J. Trupl, M. Liskova. Fungemia in neonates: Report of 80 cases from seven university hospitals, Pediatrics 105 (2000) 913-914.

[56] T.L. Lamagni, B.G. Evans, M. Shigematsu, E.M. Johnson. Emerging trends in the epidemiology of invasive mycoses in England and Wales (1990-9), Epidemiol. Infect. 126 (2001) 397-414.

[57] M.A. Pfaller. Nosocomial candidiasis: Emerging species, reservoirs, and modes of transmission, Clin. Infect. Dis. 22 Suppl 2 (1996) S89-94.

[58] J. R. Harlan, The Living Fields, Cambridge University Press (1995).
[59] E. C. Oerke, H.W. Dehne, F. Schönbeck, A. Weber, Crop Production and Crop Protection—Estimated Losses in Major Food and Cash Crops, Elsevier, Amsterdam, (1994) pp 808.
[60] V. Gewin, Bioterrorism: agricultural shock, Nature 421 (2003) 106.
[61] Anonymous, Sugar Cane Orange Rust in Australia. Int. Sugar J. 103 (2001) 146.
[62] M.G. Milgroom, K. Wang, Y. Zhou, S.E. Lipari, S. Kaneko, Intercontinental population structure of the chestnut blight fungus, *Cryphonectria parasitica*, Mycologia 88 (1996) 179.
[63] S.L. Anagnostakis, American chestnut sprout survival with biological control of the chestnut-blight population, For. Ecol. Manage. 152 (2001) 225.
[64] C.M. Brasier, Rapid evolution of introduced plant pathogens via interspecific hybridization, Bioscience 51 (2001)123.
[65] P.K. Anderson, A.A. Cunningham, N.G. Patel, F.J. Morales, P.R. Epstein, P. Daszak, Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol. Evol. 19 (2004) 535.
[66] P. van West, A.A. Appiah, N.A.R. Gow. Advances in research on oomycete root pathogens, Phys. Mol. Plant Path. 62 (2003) 99.
[67] J.S. Niederhauser, The potato association of America and international cooperation 1916-1991, Am. Potato J. 68 (1991) 237.
[68] C. Woodham-Smith, The Great Hunter: Ireland 1845-1849, Harper and Row (1962).
[69] S.B. Goodwin, Panglobal distribution of a single clonal lineage of the Irish potato famine fungus, Proc. Natl. Acad. Sci. USA, 91 (1994) 11591.
[70] S.B. Goodwin, et al., Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus, Phytopathology 85 (1995) 669.
[71] R. Edwards, Tomorrow's bitter harvest – the genetic diversity of our agriculture is rapidly vanishing, leaving our crops prone to pest and plague, New Sci. August 17, (1996) pp. 14–15.
[72] D.M. Rizzo, M. Garbelotto, Sudden oak death: endangering California and Oregon forest ecosystems, Front. Ecol. Environ. 1 (2003) 197–204.
[73] R.T. Wills, The ecological impact of *Phytophthora cinnamomi* inthe Stirling Range National Park, Western-Australia. Aust. J. Ecol.18 (1993) 145–159.
[74] D.M. Kuhn, M.A. Ghanoum, Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective, Clin Microbiol Rev. 16 (2003) 144–172.
[75] A.A. Woodcock, N. Steel, C.B. Moore, S.J. Howard, A. Custovic, D.W. Denning, Fungal contamination of bedding. Allergy 61 (2006) 140.
[76] D. Sanglard, J. Bille, Current understanding of the modes of action of and resistance mechanisms to conventional and emerging antifungal agents for treatment of Candida infection. In: *Candida* and Candidiasis (Eds R.A. Calderone) (2002) ASM Press, Washington. pp 349.
[77] J. Bratjburg, W.G. Powderly, G.S. Kobayashi, G. Medoff, Amphotericin B: current understanding of mechanisms of action, Antimicrob. Agents Chemother. 34 (1999) 183.
[78] J. Adler-Moore, AmBisome targeting to fungal infections, Bone Marrow Transl. 14 (1994) S3.

[79] R.P. Rapp, P.O. Gubbins, M.E. Evans, Amphotericin B lipid complex, Ann. Pharmacother. 31 (1997) 1174.

[80] J. Bolard, Mechanisms of action of anti-candida drug: amphotericin B and its derivatives. In: *Candida albicans*, cellular and molecular biology (eds. R. Prasad). Springer-Verlag, Germany (1991) p213.

[81] F. Peyron, A. Favel, A. Michel-Nguyen, M. Gilly, P. Regli, A. Bolmstrom, Improved detection of amphotericin B-resistant isolates of *Candida lusitaniae* by E-Test, J. Clin. Microbiol. 39 (2001) 339.

[82] J. Bolard, J. Milhaud, Interaction of the anti-*Candida* amphotericin B (and other polyene antibiotics) with lipids. In: Lipids of pathogenic fungi (eds R. Prasad, M. Ghannoum). CRC Press, (1996) p253.

[83] P. Aramwit, B.G. Yu, A. Lavasanifar, J. Samuel, G.S. Kwon, The effect of serum albumin on the aggregation state and toxicity of amphotericin B, J. Pharm Sci 89 (2000) 1589.

[84] S.L. Kelly, D.C. Lamb, D.E. Kelly, J. Loeffler, H. Einsele, Resistance to fluconazole and amphotericin in *Candida albicans* from AIDS patients, Lancet 348 (1996) 1523.

[85] F.S. Nolte, T. Parkinson, D.J. Falconer, S. Dix, J. Williams, C. Gilmore, R. Geller, J.R. Wingard, Isolation and characterization of fluconazole-and amphotericin β-resistant *Candida albicans* from blood of two patients with leukemia, Antimicrob. Agents. Chemother. 44 (1997)196.

[86] M.L. Sokol-Anderson, J. Brajtburg, G. Medoff, Amphotericin B-induced oxidative damage and killing of *Candida albicans*, J Infect Dis. 154 (1986) 76.

[87] H. Vanden Bossche, P. Marichal, F.C. Odds, Molecular mechanisms of drug resistance in fungi, Trends Microbiol. 2 (1994) 393.

[88] S.L. Kelly, D.C. Lamb, B.C. Baldwin, A.J. Corran, D.E. Kelly, Characterization of *Saccharomyces cerevisiae* CYP61, sterol Δ22-saturase, and inhibition by azole antifungal agents, J. Biol Chem. 27 (1997) 9986.

[89] D.C. Coleman, M.G. Rinaldi, K.A. Haynes, J.H. Rex, R.C. Summerbell, E.J. Anaissie, A. Li, D.J. Sullivan, Importance of *Candida* species other than *Candida albicans* as opportunistic pathogens, Med. Mycol. 36 (1998) 156.

[90] A.H. Groll, S.C. Piscitelli, T.J. Walsh, Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development, Adv. Pharmacol. 44 (1998) 343.

[91] C. Onyewu, J.R. Blankenship, M. Del Poeta, J. Heitman, Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against *Candida albicans*, *Candida glabrata*, and *Candida krusei*, Antimicrob. Agents Chemother. 47 (2003) 956.

[92] S. Tawara, F. Ikeda, K. Maki, Y. Morishita, K. Otomo, N. Teratani, N. et al. *In vitro* activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi, Antimicrob. Agents Chemother. 44 (2000) 57.

[93] Z. Ma, T.J. Michailides, Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi, *Crop Protection* 24 (2005) 853-863.

[94] Y. Kurahashi, T. Hattori, S. Kagabu, R. Pontzen, Mode of action of the novel rice blast fungicide KTU 3616, Pestic. Sci. 47 (1996) 199.

[95] E. Sieverding,T. Hirooka, T. Nishiguchi, Y. Yamamoto, V.J. Spadafora, H. Hasui, AC 382042 — a new rice blast fungicide, Proceedings of the Brighton Crop Protection Conference, Pests and Diseases, (1998) 359.

[96] M.H. Nguyen , J.E. Peacock, A.J. Morris, D.C. Tanner, M.L. Nguyen, D.R. Snydman, M.M. Wagener, M.G. Rinaldi, V.L. Yu, The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance, Am J Med. 100 (1996) 617.

[97] D. Abi-Said, E. Anaissie, O. Uzun, I. Raad, H. Pinzcowski, S. Vartivarian, The epidemiology of hematogenous candidiasis caused by different Candida species, Clin. Infect. Dis. 24 (1997) 1122.

[98] R.S. Liao, R.P. Rennie, J.A. Talbot, Assessment of the effect of amphotericin B on the vitality of *Candida albicans*, Antimicrob Agents Chemother 43 (1999) 1034.

[99] S.A. Mousavi, G.D. Robson, Oxidative and amphotericin B-mediated cell death in the opportunistic pathogen *Aspergillus fumigatus* is associated with an apoptotic-like phenotype. Microbiology 150 (2004) 1937-1945.

[100] T. Oki, M. Konishi, K. Tomatsu, K. Saitoh, M.Tsunakawa, M. Nishio, T. Miyaki, H. Kawaguchi, Pradimicin, a novel class of potent antifungal antibiotics, J. Antibiot. (Tokyo), 41 (1988) 1701.

[101] F. Hiramoto, N. Nomura T. Furumai, T. Oki, Y. Igarashi, Apoptosis-like cell death of *Saccharomyces cerevisiae* induced by a mannose-binding antifungal antibiotic, pradimicin, J Antibiot (Tokyo) 56 (2003) 768.

[102] B. Coyle, P. Kinsella, M. McCann, M. Devereux, R. O'Connor, M. Clynes, K. Kavanagh, Induction of apoptosis in yeast and mammalian cells by exposure to 1,10-phenanthroline metal complexes, Toxicol In Vitro 18 (2004) 63-70.

[103] M. McCann, M. Geraghty, M. Devereux, D. O'Shea, J. Mason, L. O'Sullivan, Insights into the mode of action of the anti-*Candida* activity of 1,10-phenanthroline and its metal chelates. Metal-Based Drugs 7 (2000) 185–193.

[104] M.L. Narasimhan, B. Damsz, M.A. Coca, J.I. Ibeas, D.J. Yun, J.M. Pardo, P.M. Hasegawa, R.A. Bressan, A plant defense response effector induces microbial apoptosis, Mol Cell 8 (2001) 921-930.

[105] E. Leiter, H. Szappanos, C. Oberparleiter, L. Kaiserer, L. Csernoch, T. Pusztahelyi *et al*.,Antifungal protein PAF severely affects the integrity of the plasmamembrane of *Aspergillus nidulans* amd induces an apoptosis-like phenotype, Antimicrob. Agents Chemother. 49 (2005) 2445.

[106] I. Ivanowska, J.M. Hardwick, Viruses activate a genetically conserved cell death pathway in a unicellular organism, J. Cell Biol. 170 (2005) 391.

[107] C.O. Morton, A. Hayes, M. Wilson, B.M. Rash, S.G. Oliver, P. Coote, Global Phenotype Screening and Transcript Analysis Outlines the Inhibitory Mode(s) of Action of Two Amphibian-Derived, {alpha}-Helical, Cationic Peptides on *Saccharomyces cerevisiae*, Antimicrob. Agents Chemother. 51 (2007) 3948.

[108] J. Reiter, E. Herker, F. Madeo, M.J. Schmitt, Viral killer toxins induce caspase-mediated apoptosis in yeast, J. Cell Biol. 168 (2005) 353.

[109] A.M. Aerts, I.E.J.A. François, E.M.K. Meert, Q-T. Li, B.P.A. Cammue, K. Thevissen, The Antifungal Activity of RsAFP2, a plant defensin from *Raphanus sativus*, involves the induction of reactive oxygen species in *Candida albicans*, J. Mol. Microbiol. Biotechnol. 13 (2007) 243.

[110] D. Wunder, J. Dong, D. Baev, M. Edgerton, Human salivary histatin 5 fungicidal action does not induce programmed cell death pathways in *Candida albicans*, Antimicrob Agents Chemother 48 (2004) 110-115.

[111] H.J. Helmerhorst, R.F. Troxler, F.G. Oppenheim, The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species, Proc Nat. Acad Sci.USA 98 (2001) 14637–14642.

[112] J. Cheng, T.S. Park, L.C. Chio, A.S. Fischl, X.S. Ye, Induction of apoptosis by sphingoid long-chain bases in *Aspergillus nidulans*, Mol Cell Biol 23 (2003) 163-177.

[113] D.P. Kontoyiannis, P.J. Murray, Fluconazole toxicity is independent of oxidative stress and apoptotic effector mechanisms in *Saccharomyces cerevisiae*, Mycoses 46 (2003) 183-186.

[114] E. Dadachova, R.W. Howell, R.A. Bryan, A. Frenkel, J.D. Nosanchuk, A. Casadevall, Susceptibility of the human pathogenic fungi *Cryptococcus neoformans* and *Histoplasma capsulatum* to γ-radiation versus radioimmunotherapy with α- and β-emitting radioisotopes, J. Nucl. Med. 45 (2003) 313.

[115] S. Barhoom, A. Sharon, Bcl-2 proteins link programmed cell death with growth and morphogenetic adaptations in the fungal plant pathogen *Colletotrichum gloeosporioides,* Fungal Genet. Biol. 44 (2006) 34.

[116] P. Fabrizio, V.D. Longo, The chronological life span of *Saccharomyces cerevisiae*, Aging Cell 2 (2003) 73-81.

[117] S. Biella, M.L. Smith, J.R. Aist, P. Cortesi, M.G. Milgroom, Programmed cell death correlates with virus transmission in a filamentous fungus, Proc. R. Soc. Lond. B 269 (2002) 2269-2276.

[118] T.D. Allen, A.L. Dawe, D.L. Nuss, Use of cDNA microarrays to monitor transcriptional responses of the chestnut blight fungus *Cryphonectria parasitica* to infection by virulence-attenuating hypoviruses, Euk. Cell 2 (2003) 1253-1265.

[119] B. Chen, S. Gao, G.H. Choi, D.L. Nuss, Extensive alteration of fungal gene transcript accumulation and elevation of G-protein-regulated cAMP levels by a virulence-attenuating hypovirus, Proc. Natl. Acad. Sci. USA 93 (1996) 7996-8000.

[120] C. Chen, M.B. Dickman, Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Proc. Natl. Acad. Sci. USA 102 (2005) 3459.

[121] A.J. Phillips, J.D. Crowe, M. Ramsdale, Ras pathway signaling accelerates programmed cell death in the pathogenic fungus *Candida albicans*, Proc. Natl. Acad. Sci. USA 103 (2006) 726.

[122] A. Stanhill, N. Schick, D. Engelberg, The yeast Ras/cyclic AMP pathway induces invasive growth by suppressing the cellular stress response. Mol Cell Biol 19 (1999) 7529–7538.

[123] F. Garcia-Olmedo, A. Molina, J.M. Alamillo, P. Roderiguez-Palenzuela, Plant defense peptides. Biopolymers 47 (1998) 479–491.

[124] G.S. Ali, A.S.N. Reddy, Inhibition of fungal and bacterial plant pathogens by synthetic peptides: in vitro growth inhibition, interaction between peptides and inhibition of disease progression, Mol Plant-Microbe Interact 13 (2000) 847–859.

[125] C.A. D'Souza, J. Heitman, Conserved cAMP signaling cascades regulate fungal development and virulence, FEMS Microbiol Rev 25 (2001) 349-364.

[126] J.H. Yu, J. Wieser, T.H. Adams, The *Aspergillus* FlbA RS domain protein anagonises G-protein signalling to block proliferation and allow development, EMBO J. 15 (1996) 5184.

[127] M.A. Coca, B. Damsz, D-J. Yun, P.M. Hasegawa R.A. Bressan RA, M.L. Narasimhan, Heterotrimeric G-proteins of a filamentous fungus regulate cell wall composition and susceptibility to a plant PR-5 protein, The Plant J. 22 (2000) 61.

[128] G.H. Huh, B. Damsz, T.K. Matsumoto, M.P. Reddy, A.M. Rus, J.I. Ibeas, M.L. Narasimhan, R.A. Bressan, P.M. Hasegawa, Salt causes ion disequilibrium-induced programmed cell death in yeast and plants, Plant J 29 (2002) 649-659.

[129] F.F. Severin, A.A. Hyman, Pheromone induces programmed cell death in *S. cerevisiae*, Curr Biol 12(2002) R233-R235.

[130] T. Edlind, L. Smith, K. Henry, S. Katiyar, J. Nickels, Antifungal activity in *Saccharomyces cerevisiae* is modulated by calcium signalling. Mol Microbiol 46 (2002) 257-268.

[131] D. Sanglard, F. Ischer, O. Marchetti, J. Entenza, J. Bille, Calcineurin A of *Candida albicans*: involvement in antifungal tolerance, cell morphogenesis and virulence, Mol. Microbiol. 48 (2003) 959.

[132] W.J. Steinbach, R.A. Cramer, B.Z. Perfect, C. Henn, K. Nielsen, J. Heitman, J.R. Perfect, Calcineurin inhibition or mutation enhances cell wall inhibitors against *Aspergillus fumigatus*, Antimicrob. Agents Chemother. 51 (2007) 2979.

[133] A.G. Uren, K. O'Rourke, L.A. Aravind, M.T. Pisabarro, S. Seshagiri, E.V. Koonin, V.M. Dixit, Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma, Mol. Cell 6 (2000) 961.

[134] N. Fedorova, J. Badger, G. Robson, J. Wortman, W. Nierman, Comparative analysis of programmed cell death pathways in filamentous fungi, BMC Genomics. 6 (2005)177.

[135] C. Thrane, U. Kaufmann, B.M. Stummann, S. Olsson, Activation of caspase-like activity and poly (ADP-ribose) polymerase degradation during sporulation in *Aspergillus nidulans*, Fungal Genet. Biol. 41 (2004) 361.

[136] M. Bettiga, L. Calzari, I. Orlandi, L. Alberghina, M. Vai, Involvement of the yeast metacaspase Yca1 in *ubp10*Δ-programmed cell death, FEMS Yeast Res 5 (2004) 141–147.

[137] M.A. Khan, P.B. Chock, E.R. Stadtman, Knockout of caspase-like gene, YCA1, abrogates apoptosis and elevates oxidized proteins in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 102 (2005) 17326–17331.

[138] M. Weinberger, L. Ramachandran, L. Feng, K. Sharma, X. Sun, M. Marchetti, M., et al. (2005) Apoptosis in budding yeast caused by defects in initiation of DNA replication, J Cell Sci 118: 3543–3553.

[139] Maeta, K., Izawa, S., and Inoue, Y. (2005) Methylglyoxal, a metabolite derived from glycolysis, functions as a signal initiator of the high osmolarity glycerol-mitogen-activated protein kinase cascade and calcineurin/Crz1-mediated pathway in *Saccharomyces cerevisiae*, J Biol Chem 280: 253–260.

[140] P. Hauptmann, C. Riel, L.A. Kunz-Schughart, K.U. Frohlich, F. Madeo, L. Lehle, Defects in N-glycosylation induce apoptosis in yeast, Mol Microbiol 59 (2006) 765–778.

[141] R.J. Howard, M.A. Ferrari, D.H. Roach and N.P. Money, Penetration of hard substrates by a fungus employing enormous turgor pressures, Proc. Natl. Acad. Sci. USA 88 (1991) 11281–11284

[142] J.D. de Jong, B.J. McCormack, N. Smirnoff, N.J. Talbot, Glycerol generates turgor in rice blast, Nature 389 (1997) 244-245.

[143] D. Moore, Programmed cell death alive and well in fungi. Mycol Res 107 (2004) 1251.

[144] K. Wolfe, Comparative genomics and genome evolution in yeasts. Phil. Trans. Royal Soc. B 361 (2006) 403.

[145] M. Liu, M.D. Healy, B.A. Dougherty, K.M. Esposito, T.C. Maurice, C.E. Mazzucco, R.E. Bruccoleri, D.B. Davison, M. Frosco, J.F. Barrett, Y-K. Wang, Conserved fungal genes as potential targets for broad-spectrum antifungal drug discovery, Euk. Cell 5 (2006) 638-649 .

[146] T. Roemer *et al.*, Large-scale essential gene identification in *Candida albicans* and applications to antifungal drug discovery, Mol. Micro. 50 (2003) 167–181.

[147] M. Weig, A.J.P. Brown, Genomics and the development of new diagnostics and anti-*Candida* drugs, Trends in Microbiology 15 (2007) 310-317.

[148] B. Ink, M. Zornig, B. Baum, N. Hajibagheri, C. James, T. Chittenden, G. Evan, Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells, Mol Cell Biol 17 (1997) 2468-2474.

[149] J.M. Jurgensmeier, S. Krajewski, R.C. Armstrong, G.M. Wilson, T. Oltersdorf, L.C. Fritz, J.C. Reed, S. Ottilie, Bax- and Bak-induced cell death in the fission yeast *Schizosaccharomyces pombe*, Mol Biol Cell 8 (1997) 325-339

[150] M. Ligr, F. Madeo, E. Frohlich, W. Hilt, K.U. Frohlich, D.H. Wolf, Mammalian Bax triggers apoptotic changes in yeast, FEBS Lett. 438 (1998) 61-65.

[151] K. De Smet, I. Eberhardt, R. Reekmans, R. Contreras, Bax-induced cell death in *Candida albicans*, Yeast 21 (2004) 1325.

[152] W. Martinet, D. Van den Plas, H. Raes, R. Reekmans, R. Contreras, Bax-induced cell death in *Pichia pastoris*, Biotechnology Lett 21 (1999) 821-829.

[153] M.D. Rose, G.R. Fink, *KAR1*, a gene required for function of both intranuclear and extranuclear microtubules in yeast, Cell 48 (1987) 1047-1060.

[154] D. Burke, P. Gasdaska, L. Hartwell, Dominant effects of tubulin overexpression in *Saccharomyces cerevisiae*, Mol Cell Biol 9 (1989) 1049-1059.

[155] V. Berlin, C.A. Styles, G.R. Fink, *BIK1*, a protein required for microtubule function during mating and mitosis in *Saccharomyces cerevisiae*, colocalizes with tubulin, J Cell Biol 111 (1990) 2573-2586.

[156] V. Magdolen, D.G. Drubin, G. Mages, W. Bandlow, High levels of profilin suppress the lethality caused by overproduction of actin in yeast cells, FEBS Lett 316 (1993) 41-47.

[157] P. Russell, P. Nurse, Negative regulation of mitosis by *wee1*$^{+}$,a gene encoding a protein kinase homolog. Cell 49 (1987) 559-567.

[158] S.A. Osmani, R.T. Pu, N.R. Morris, Mitotic induction and maintenance by overexpression of a G2-specfic gene that encodes a potential protein kinase. Cell 53 (1988) 237-244.

[159] M. Whiteway, L. Hougan, D.Y. Thomas, Overexpression of the *STE4* gene leads to mating response in haploid *Saccharomyces cerevisiae*. Mol Cell Biol 10 (1990) 217-222.

[160] J.B. Millar, P. Russell, J.E. Dixon, K.L. Guan, Negative regulation of mitosis by two functionally overlapping PTPases in fission yeast. EMBO J 11 (1992) 4943-4952.

[161] S. Ottilie, J. Chernoff, G. Hannig, C.S. Hoffman, R.L. Erikson, The fission yeast genes *pyp1*+ and *pyp2*+ encode protein tyrosine phosphatases that negatively regulate mitosis, Mol Cell Biol 12 (1992) 5571-5580.

[162] H. Liu, J. Krizek, A. Bretscher, Construction of a *GAL1*-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast, Genetics 132 (1992) 665-673.

[163] S.W. Ramer, S.J. Elledge, R.W. Davis, Dominant genetics using a yeast genomic library under the control of a strong inducible promoter, Proc. Natl. Acad. Sci. USA 89 (1992) 11589-11593.

[164] C. Espinet, M.A. de la Torre, M. Aldea, E. Herrero, An efficient method to isolate yeast genes causing overexpression-mediated growth arrest, Yeast 11 (1995) 25-32.

[165] R. Akada, J. Yamamoto, I. Yamashita, Screening and identification of yeast sequences that cause growth inhibition when overexpressed, Mol Gen Genet 254 (1997) 267-274.

[166] L. Romani, Innate and adaptive immunity to systemic *C. albicans* infection. In: Fungal Immunology from an organ perspective. (Eds. P.L. Fidel and G.B. Huffnagle). Springer, New York. (2005) p 377.

[167] A. Mullick, M. Elias, P. Harakidas, A. Marcil, M. Whiteway, B. Ge, T. J. Hudson, A. Caron, L. Bourget, S. Picard, O. Jovcevski, B. Massie, D. Thomas. Gene expression in HL60 granulocytoids and human polymorphonuclear leukocytes exposed to *Candida albicans*, Infect. Immun. 72 (2004) 414.

[168] C. Fradin, P. De Groot, D. MacCallum, M. Schaller, F. Klis, F. Odds, B. Hube. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood, Mol. Microbiol. 56 (2005) 397.

[169] C. Fradin, A.L. Mavor, G. Weindl, M. Schaller, K. Hanke,S.H.E. Kaufmann, H. Mollenkopf, B. Hube. The early transcriptional response of human granulocytes to infection with *Candida albicans* is not essential for killing but reflects cellular communications, Infect Immun. 75 (2007) 1493.

[170] I. Rubin-Bejerano, I. Fraser, P. Grisafi, G. Fink. Phagocytosis by neutrophils induces an amino acid deprivation response in *Saccharomyces cerevisiae* and *Candida albicans*, Proc. Natl. Acad. Sci. USA 100 (2003) 11007.

[171] M.C. Lorenz, J.A. Bender, G.R. Fink. Transcriptional response of *Candida albicans* upon internalization by macrophages, Eukaryot Cell. 3 (2004) 1076.

[172] E. Fernández-Arenas, V. Cabezón , C. Bermejo, J. Arroyo, C. Nombela, R. Diez-Orejas, C. Gil, Integrated proteomic and genomic strategies bring new insight into *Candida albicans* response upon macrophage interaction, Mol Cell Proteomics. 6 (2006) 460.

[173] Crop Life International. Annual Report 2006/2007. Global market performance. p1-26.

[174] Business Communication Company Reports. Global market for antifungal agents. p1-133 available at http://www.piribo.com/publications/therapeutic/global_markets_antifungal_agents.html.